TOXICOLOGICAL ASSESSMENT OF COMBINED FUNGICIDES PROPOSED FOR APPLICATION ON CEREAL SPIKED CROPS IN UKRAINE

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Abstract: Toxicological assessment of combined fungicides proposed for application on cereal spiked crops in Ukraine was performed according approved State Sanitary Norms and Rules. Statistics, bibliographical methods were used in the study. The classes of hazard were established after evaluation of toxicometry parameters, which included assessment of acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, irritation potential to eyes (mucous membranes) and skin, and sensitizing properties. Integral hazard class of the studied formulations was established. Formulations No. 1–3, 5 and 7 can be pertained to integral hazard class 2 by acute inhalation toxicity as limiting criterion of hazard (formulation No. 1 also has additional point of concern – its irritation of the skin and eyes). Formulation No.4, 6 and 8 were classified as integral hazard class 3 by the same limiting criterion of hazard, but the danger of formulation No. 4 possibility to cause sensitization and irritation of the eyes was taken into account. Recommendations for the studied combined fungicides application in agricultural sector of Ukraine were given. Maximum daily time of contact with formulations for workers engaged in its application on cereal spiked crops was suggested. 4 hours per day were approved for formulations No. 1–3, 5 and 7, and 6 hours per day for No. 6 and 8, respectively.
Keywords: combined fungicides, acute oral, dermal, inhalation toxicity, irritation potential, and sensitization.

1. Introduction

Estimation of toxic properties of active substances and pesticidal formulations on its basis is the one of the most important stages of registration studies. Assessment of the safety of plant protection chemicals in Ukraine is carried out on the basis of a systematic approach using the principles of the modern approved toxicological classification of pesticides [1]. The integral safety class of a specific formulation is established on the basis of hygienic and toxicometric indices as well as long-term exposure indices taking into account the limiting criteria of hazard. It is the results of toxicological studies that are the basis for the development of hygienic standards and preventive measures that can guarantee safety for the population and workers engaged in application of these substances.

The aim of the study is to carry out toxicological assessment of combined fungicides proposed for application on cereal spiked crops in Ukraine.

The task of the study is to perform evaluation of the formulations safety based on the induced toxic effects in mammals.

2. Methods

Department of hygiene and ecology No. 1 and Institute of hygiene and ecology of Bogomolets National medical University was the base for research.

The studies were conducted in 2015–2017.

Toxicological, statistics, bibliographical methods were used in the study. Evaluation of the formulation safety was carried out based on the induced toxic effects in mammals, and after we carried out a comparative toxicological evaluation. In the course of our research, we studied fungicides on the basis of active substances with the physicochemical properties listed in **Table 1**.

The content of active substances in the formulations studied is shown in **Table 2**.

The studied effects of combined fungicides (formulations No.1–8) were: acute oral toxicity (LD_{50}, per os mg/kg), acute dermal toxicity (LD_{50}, dermal, mg/kg) and acute inhalation toxicity (LC_{50}, inhalation, mg/l, time of exposure 4 hours (Wistar rats), as well as skin and eye irritation properties (New Zealand rabbits), and skin sensitization (guinea pigs) according to [13].

3. Results

Findings were represented in the **Table 3**.

All the studied combined pesticides were classified as hazard class 4 by acute oral toxicity (except formulation No. 5 – hazard class 3). Formulations No. 1, 3, 6 were classified as hazard class 4 by acute dermal toxicity, and formulations No. 2, 4, 5, 7, 8 are of hazard class 3. Formulations No.4, 6, 8 were classified as hazard class 3 by acute inhalation toxicity (4-hour exposure), and formulations No. 1–3, 5, 7 are of hazard class 2.

Formulations No. 2– 4, 7, 8 are not irritants of the skin and thus were classified as hazard class 4, meanwhile formulations No. 5, 6 mildly irritating skin (hazard class 3), and formulation No. 1 moderately irritating the skin and was pertained to hazard class 2. Formulations No. 3 and 7 are not irritants of the mucous membranes (eyes) and thereby pertained to hazard class 4, formulations No. 2, 5, 6, 8 mildly irritating eyes (hazard class 3), formulations No. 1 and 4 have extreme irritating potential and were classified as hazard class 1.

Almost all formulations (No. 2, 3, 5–8) are not sensitizers and were pertained to hazard class 4, but formulation No. 1 was classified as mild allergen (hazard class 3) and formulation No. 4 caused moderate sensitization and as such was classified as hazard class 2.

4. Discussion

Integral hazard class of the studied combined fungicides was established. Formulations **No. 1–3, 5 and 7** can be pertained to integral hazard class 2 by acute inhalation toxicity as limiting criterion of hazard (formulation **No. 1** also has additional point of concern – its irritation of the skin and eyes). Formulation **No. 4, 6 and 8** were classified as integral hazard class 3 by the same limiting criterion of hazard, but the danger of formulation **No. 4** possibility to cause sensitization and irritation of the eyes was taken into account.

Abovementioned allowed us to substantiate maximum daily time of contact with formulations for workers engaged in its application on cereal spiked crops. 4 hours per day were approved for formulations **No. 1–3, 5** and **7**, and 6 hours per day for **No. 6** and **8**, respectively.

Another recommendation was given and approved for formulation **No. 4** due to its irritating and sensitizing properties and for **No. 1** due to its irritating potential (label of its should contain information and special signs on the packaging).

Table 1
Physico-chemical properties of active substances of combined fungicides

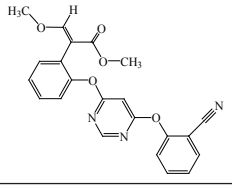
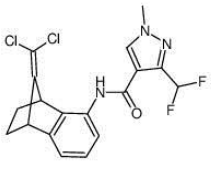
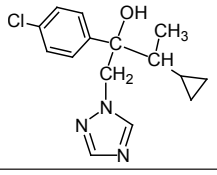
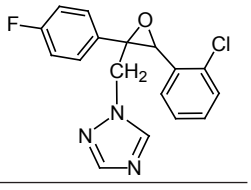
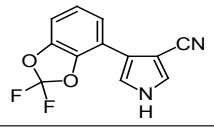
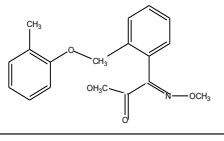
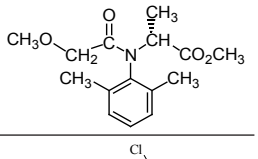
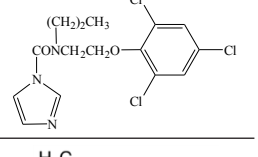
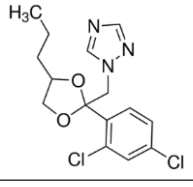
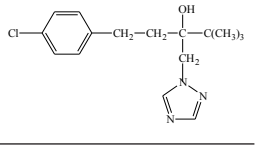
Active ingredient	Chemical class of active ingredient	Active substance minimum purity (g/kg(l)	IUPAC	Formula
Azoxystrobin [2]	methoxyacrylate strobilurin	965	methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	
Benzovindiflupyr [3, 4]	Pyrazole-carboxamide	960	N-((1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl)-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide	
Cyproconazole [5]	conazole fungicides (triazoles)	940	(2*RS*,3*RS*;2*RS*,3*SR*)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol	
Epoxiconazole [6]	conazole fungicides (triazoles)	920	(2RS,3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1H-1,2,4-triazole	
Fludioxonil [7]	pyrrole fungicides	950	4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile	
Kresoxim-methyl [8]	methoxyiminoacetate strobilurin	934	methyl (E)-methoxyimino[α-(o-tolyloxy)-o-tolyl] acetate	
Metalaxyl-M [9]	acylamino acid	910	methyl N-(methoxyacetyl)-N-(2,6-xylyl)-D-alaninate	
Prochloraz [10]	amide, (imidazoles)	>960	N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl] imidazole-1-carboxamide	
Propiconazole [11]	conazole fungicides (triazoles)	930	(2RS,4RS;2RS,4SR)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole	
Tebuconazole [12]	conazole fungicides (triazoles)	950	(RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol	

Table 2
The composition of the studied combined fungicides (Formulations No. 1–8)

Formulation	Active ingridients	Concentration (g/l)	Formulation	Active ingridients	Concentration (g/l)
No. 1	azoxystrobin	200	No. 5	azoxystrobin	120
	cyproconazole	80		tebuconazol	200
No. 2	epoxiconazole	75	No. 6	fludioxonil	18,7
	prochloraz	300		cyproconazole	6,25
No. 3	kresoxim-methyl	125	No. 7	azoxystrobin	80
	tebuconazol	240		tebuconazol	160
No. 4	benzovindiflupyr	83,33	No. 8	metalaxyl-M	20
	cyproconazole	66,67		tebuconazol	30
	propiconazole	208,33			

Table 3
Toxicometric parameters of combined fungicides (Formulations No. 1–8)

Studied effect	Animal species	Dose (effect) [class of hazard according State Sanitary Rules and Norms 8.8.1.002-98]			
		No. 1	No. 2	No. 3	No. 4
LD50, per os mg/kg	Wistar rats	f>2000 [4]	>2000 [4]	>5050 [4]	>2000 [4]
LD50, dermal mg/kg	Wistar rats	>4000 [4]	>2000 [3]	>5050 [4]	>2000 [3]
LC50, inhalation mg/l 4 h.	Wistar rats	>1868 [2]	>2500 [2]	– [2]	>5080 [3]
Skin irritant	New Zealand rabbits	moderate irritant [2]	non irritant [4]	non irritant [4]	non irritant [4]
Eye irritant	New Zealand rabbits	extreme irritant [1]	mild irritant [3]	non irritant [4]	extreme irritant [1]
Skin sensitizer	Guinea pig	mild allergen [3]	non sensitizer [4]	non sensitizer [4]	moderate allergen [2]
Studied effect	Animal species	Dose (effect) [class of hazard according State Sanitary Rules and Norms 8.8.1.002-98]			
		No. 5	No. 6	No. 7	No. 8
LD50, per os mg/kg	Wistar rats	>300–<2000 [3]	>3000 [4]	f>2000 [4]	>2000 [4]
LD50, dermal mg/kg	Wistar rats	>2000 [3]	>4000 [4]	>2000 [3]	>2000 [3]
LC50, inhalation mg/l 4 h.	Wistar rats	>4790 [2]	– [3]	>890 [2]	>4110 [3]
Skin irritant	New Zealand rabbits	mild irritant [3]	mild irritant [3]	non irritant [4]	non irritant [4]
Eye irritant	New Zealand rabbits	mild irritant [3]	mild irritant [3]	non irritant [4]	non irritant [3]
Skin sensitizer	Guinea pig	non sensitizer [4]	non sensitizer [4]	non sensitizer [4]	non sensitizer [4]

***Note**: «–» was not studied due to physicochemical properties (class of hazard was established by active substances class)*

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